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Variability in the α and β anomer content of commercially available lactose

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Abstract

Lactose, a disaccharide is a ubiquitous excipient in many pharmaceutical formulations which exists in two anomeric forms; either as α- or β-lactose. The anomers have different properties which can affect their application. Nevertheless, batches of lactose products are widely produced by many manufacturers, and is available in many grades. However, the anomeric content of these batches has not been accurately characterized and reported previously. Therefore, the aim of this study was to analyse a set of 19 commercially available samples of lactose using a novel H^1-NMR technique to establish a library showing the anomeric content of a large range of lactose products. The lactose samples were also analysed by DSC. The anomeric content of the α-lactose monohydrate samples were found to vary by more than 10 %, which might influence bioavailability from final formulations. The data showed that there is a need to determine and monitor the anomeric content of lactose and this should be a priority to both manufacturers and formulators of medicines.

Keywords:

Lactose (grades); anomer; α-lactose, β-lactose, NMR; DSC; excipient; epimerisation/mutarotation; survey

1 Introduction

1.1 Lactose

Lactose is a disaccharide formed through a β-1,4-glycosidic bond between α/β-D-glucose and β-D-galactose (Berg et al., 2002; Jawad et al., 2012). It has many uses in pharmaceutical formulations, with its advantageous properties including: low cost, inert nature, safety and low hygroscopicity (Jivraj et al., 2000). It is used extensively in tablet formulations as a bulking agent, being present in an estimated 70% of commercially available tablets (Illanes, 2016). Lactose is often incorporated in dry powder inhalers (DPIs) where it can fulfill the triple function of bulking agent, flow aid and carrier particle (Chow et al., 2007; Kinnunen et al., 2014). Approximately 6 million tons of lactose are produced yearly to supply the worldwide market (Geiger et al., 2016).

Lactose has two anomeric forms, termed α and β. These forms exist as a consequence of an orientational change of the hydrogen and hydroxyl groups around the chiral centre on the C1 carbon (Jawad et al., 2014). They have different non-superimposable chemical structures that could be considered as different chemical species.

1.2 Lactose anomers

Lactose is typically isolated from whey, which is produced as a byproduct from cheese-making (Keri Marshall, 2004). Several crystallization steps are used to purify the end product. When crystallization is carried out at high temperatures (93.5°C or above) the sample contains a high proportion of anhydrous β-lactose crystals; whereas α-lactose monohydrate crystals are predominantly obtained if crystallization is conducted closer to room temperature (Wade and Weller, 1994). Thus, the distinct molecular structures associated with these anomers of lactose leads to the generation of a range of solid forms for lactose, which possess different physiochemical properties (Pharma, 2017). For example, anhydrous crystalline β-lactose has a solubility in water that is seven times that of crystalline α-lactose monohydrate in water (Illanes, 2016).

As a consequence, the form of lactose might be crucial to the efficiency and bioavailability of the active pharmaceutical ingredient in both medicines containing lactose (Tsujikawa et al., 2016) and even tablet formulations of drugs of abuse (Wawer, 2008). In tablets, the role of lactose is not only to serve as a bulking agent (to ensure uniformity of dose) but also to

improve the physical strength of tablets, and it is used for the same purposes by illicit tablet manufacturers (Chalmers et al., 2012).

Commercially available lactose powders that are used in tablets may show a range of anomeric compositions spanning those that are composed predominantly of α-lactose through to those comprising predominantly β-lactose (Pharma, 2017). The solid forms that these anomers may adopt adds another layer of complexity, for example the different anomers may be isolated as amorphous solids or under specific conditions they may combine to form anomeric co-crystals (Simpson et al., 1982). The solid forms in which lactose can exist are summarized below in Figure 1.

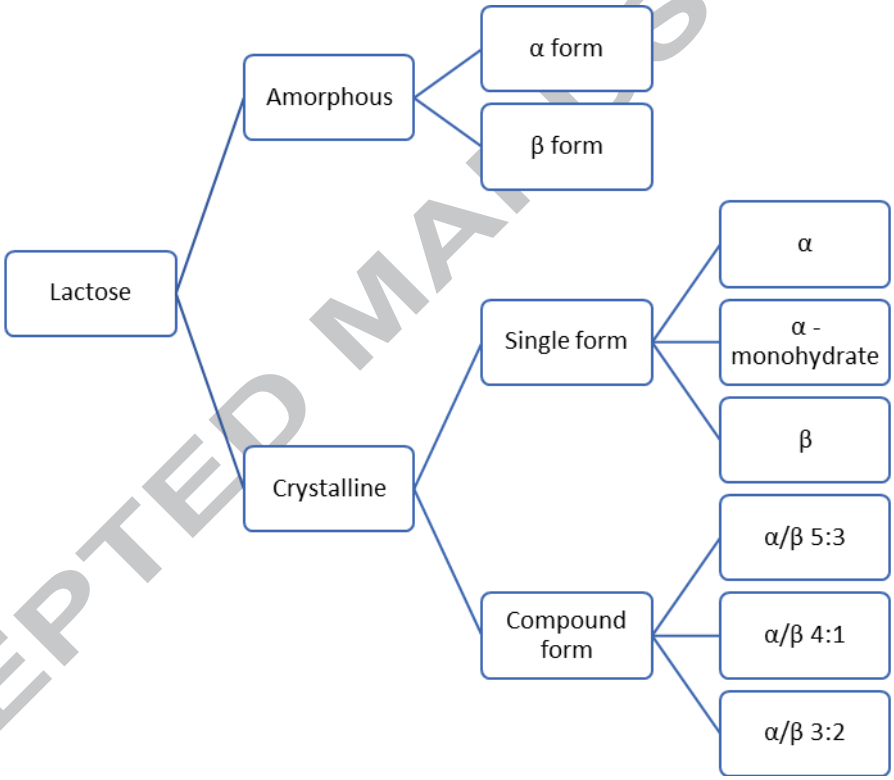


Figure 1 The solid forms of lactose available for manufacture (Adapted from Jawad et al., 2015 with permission)

Crystalline α-lactose monohydrate is one of the most stable forms of lactose but it can be dehydrated into both stable and an unstable anhydrous forms of α-lactose by the application of heat (Alderborn, 2002). In contrast, β-lactose only forms an anhydrous product and this form of lactose can be more favourable than other lactose forms because of its compression properties (Jawad et al., 2015). However, 'anhydrous β-lactose' typically contains 15% w/w of anhydrous α-lactose as an impurity. In fact, it is probable that most lactose products will contain an impurity of the anomer other than that stated within the product descriptor.

An important mechanism of lactose is called mutarotation where α- and β-lactose anomeric forms interchange in a solution until a temperature-sensitive equilibrium is established. This is considered when processing lactose as some preparation methods require lactose to be in a solution temporarily such as in spray-drying. A study also found that mutarotation may occur in solid state samples that are exposed to high humidity and temperature (Altamimi et al., 2017).

The mutarotation of lactose follows the same mechanics as the mutarotation of related sugars such as glucose (Silva et al., 2006) and has been described in detail elsewhere (Jawad et al., 2012). This occurs through the formation of a free aldehyde form from the glucose molecule in the lactose structure (

Figure 2). The aldehyde forms as a result of a protonation of the oxygen (O5 in

Figure 2), which precedes the breakage of the hydrogen bond of the O1 (Altamimi et al., 2017; Jawad et al., 2012; Lefort et al., 2006). In solution, the two forms interconvert until they reach an equilibrium depending on the pH and temperature of the solution (

Figure 2).

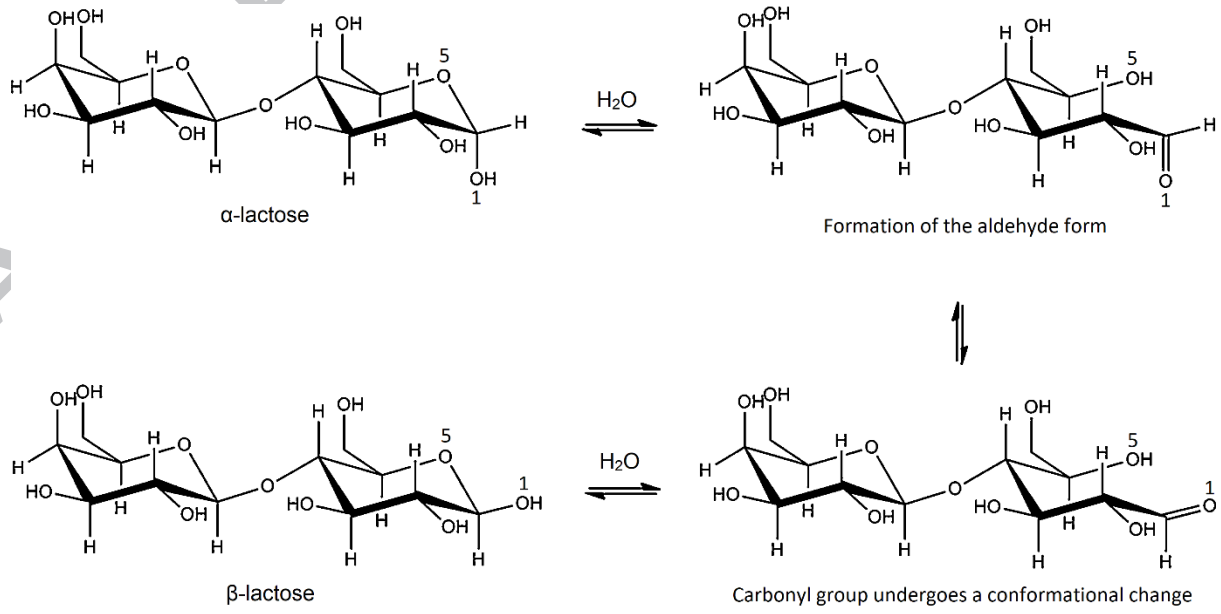


Figure 2 The forms of lactose that exist during mutarotation

In lactose containing products, the anomeric content is very often scarcely reported. Even the European Pharmacopoeia does not consider anomeric content reporting as an obligatory requirement to include in the specifications of lactose products (Rowe et al., 2006). The crystallinity or amorphous content of lactose batches has been reported extensively in previous studies (Altamimi et al., 2017; GombÁs et al., 2002; Islam and Langrish, 2010; Jawad et al., 2014; Jawad et al., 2012; Jawad et al., 2015). However, the anomeric composition of the wide range of commercially available lactose powders has not been investigated and there appears to be a clear need to develop rapid methods for this purpose.

1.3 Analytical methods used to determine the solid anomeric form of lactose powders

A variety of analytical techniques have been used to determine the presence and the form of lactose for the quality control of pharmaceuticals and have the potential to be employed in forensic science. Although nearly all of these techniques are sensitive to the anomeric composition of lactose, for reasons of signal complexity, run time, sample presentation and detection limits, none have been employed to characterize the variation in anomeric content of the broad range of lactose powders that are available to the formulation scientist. Spectroscopic methods for analyzing lactose powders include near infrared reflectance spectroscopy (NIR), terahertz (THZ) spectroscopy and Raman spectroscopy (Baer et al., 2007; Chalmers et al., 2012). For example, Raman spectroscopy has been employed to distinguish successfully the difference between genuine and counterfeit tablets (Trefi et al., 2008). Powder X-ray diffraction (PXRD) can be used to identify the anomeric crystal type (Farber et al., 2003), however if the sample is not crystalline, i.e. amorphous, PXRD is unable to differentiate between the anomeric forms of lactose. Dynamic vapour sorption (DVS) is another technique that can be used to determine the amorphous content of a sample containing a mixture of crystalline and amorphous material due to changes in the sorption profile that is attributed to the amorphous content (Mackin et al., 2002). However, it should be noted that when using DVS samples with low levels of amorphous content, large errors may occur due to the surface adsorbed moisture.

In terms of pharmaceutical analysis, although these diffraction and spectroscopy-based techniques are non-destructive, they do require a powdered sample that is representative of the whole material that is being studied. Other methods have also been employed to characterize the physical properties of solid phase lactose (Table 1).

There are several issues regarding the manufacturer's specifications of anomeric content of lactose samples that are available commercially. First, even if specifications are provided, the amount of α- and β-lactose in the powder is not detailed precisely. Should the anomeric purity be mentioned, it is given as a range, for example >98% w/w, rather than a determination of the exact anomeric content (ANON, 2017). Second, the anomeric content is sensitive to the environment and can change if the products are not stored under appropriate conditions. For example, when stored at high humidity and temperature (i.e. >90% RH and 40°C) the anomeric content of crystalline β-lactose powder changes as a function of time; with an increasing percentage of α-lactose anomer forming in the powder (Altamimi et al., 2017). Due to the differences in properties between α- and β-lactose anomers, it is likely that storage conditions of lactose containing products should be controlled to prevent possible alteration in biopharmaceutical profiles of formulated medicines.

Table 1 Summary and comparison of the analytical techniques used to determine the physicochemical properties of lactose powders

Method	Physicochemical property observed	Advantages	Disadvantages	Reference
PXRD	Isotropic diffraction pattern generated by powdered crystalline material.	Non-destructive, relatively fast (20 min).	Requires 100-200 mg homogeneous crystalline sample. Sample ground into uniform powder for accuracy (may affect stability of lactose samples).	(Crisp et al., 2011; Jawad et al., 2012; Kougoulos et al., 2010)
DVS	Obtain a sorption profile of samples using a microbalance.	Accurately determine amorphous content in mixture of amorphous and crystalline material.	Low accuracy when low amorphous content in sample. Quantification not possible if the sample being investigated forms hydrates.	(Mackin et al., 2002)
Polarimetry	Measures optical rotation of sample: chiral compounds rotate plane-polarized light, so the amount of rotation will differ depending on presence & ratio of lactose anomers.	Allows determination of chemical purity and anomeric content of lactose.	Depends on several variables: e.g. temp., concentration of optical active components. A solution (aqueous) of lactose required, for epimerization to occur. Time-sensitive due to mutarotation of lactose in the aqueous sample.	(Jawad et al., 2012; Căpriță, 2014)
DSC	Analyses enthalpies of fusion & hydrate loss of samples showing melting peaks of anomers & water loss peaks from monohydrate forms of lactose.	Anomeric composition can be determined where the areas of melting peaks and loss of hydrate are used to determine the relative amounts of the crystalline forms of anomers.	Determination of amorphous content difficult due to overlap between sample melting peak & degradation. Precision depends on heating rate & amount of sample loaded within DSC. Crystalline samples required. Milling of α-lactose can cause changes in the thermogram where the peaks do not correspond to α- or β-lactose; thus, sample history is important.	(GombÁs et al., 2002; Islam and Langrish, 2010; Badal Tejedor et al., 2018)
H^1NMR	Structure interpreted from magnetic properties due to protons in sample. Resonance of hydrogen nuclei relative to a magnetic field creates resolvable peak shifts & splitting patterns.	High specificity; non-destructive; can accurately determine anomeric content of lactose, fast technique (<10 min) when dissolved in solution.	Determination of the solid phase anomeric content of lactose, needed within 20 min of preparation of solution in DMSO. Less information is detected with undissolved samples.	(Jawad et al., 2014)

C^{13} NMR (solid state)	Magnetic property of samples provides structural data representing the carbon atoms in sample. Frequency of resonance of carbon nuclei relative to a magnetic field create peak shifts and splitting patterns to resolve chemical structures.	Non-destructive, no need to make sample in aqueous solution (simple preparation).	Requires more sample than H^1 NMR due to low abundance of C^{13} isotope and longer run (~30 min) than H^1 NMR where unstable samples can undergo reactions that change structure of sample and complicate the spectra.	(Lefort et al., 2006)

DSC has been widely used to analyze lactose in terms of assessing sample crystallinity (Table 1) and in some cases to determine crystalline contaminants in anhydrous samples (Brittain and Blaine, 2018; GombÁs et al., 2002). Lactose in whey powder has been analysed using DSC, a much faster method of analysis (anomeric content determined within 2 h), in comparison to the 6 h required for a polarimeter-based method (Ross, 1978). The DSC values for anomeric content have been reported to be within 5% of those obtained using polarimetry. This scrutiny in determination of the anomeric content of lactose has not been applied routinely to pharmaceutical products despite the inherent requirement for the highest available standards to be employed for medicines and commercial applications. The main disadvantage of DSC is that it requires crystalline samples, as it measures the enthalpy of fusion associated with the resolvable melting transitions for the anomeric forms of lactose respectively. However, when non-crystalline or amorphous lactose is heated the powder passes through a glass transition and then a subsequent re-crystallization. DSC is unable to differentiate between the glass transitions and re-crystallization peaks for the different anomeric forms of lactose. Thus, if amorphous material is a significant fraction of a lactose powder, DSC is unlikely to determine an accurate anomeric content. Even with the advantages of DSC methodology, the quantitative limitations are a possible reason as to why a more sophisticated technique might be advocated for characterizing lactose within medicines. NMR has been shown to be a candidate method for this purpose (Altamimi et al., 2017).

A gas chromatography method has been outlined in the British Pharmacopoeia and is currently employed by manufacturers that attempt to determine the anomeric content of their products (ANON, 2017). The method requires the preparation of a silylation reagent to derivatize lactose samples. Next a resolution mixture must be passed through the column before the lactose samples are introduced, so as to ensure that the resolution between the peaks is adequate (>3.0). For each sample, 10 mg of lactose needs to be dissolved in 4 mL of the silylation regent before the mixture is sonicated at room temperature for 20 min. Samples of this solution (400 µL) are then transferred to new vials and 1 mL of pyridine is introduced prior to assay (ANON, 2012). In comparison, the NMR analysis method was successful in determining the anomeric content measured without requiring derivitization, resolution mixtures or waiting times as required in the GC method (Jawad et al., 2012).

C^{13} cross-polarization magic angle spinning NMR analysis of solid state lactose has also been used to differentiate between crystalline α-lactose monohydrate and stable anhydrous lactose (Lefort et al., 2006). The monohydrate form exhibits a single peak whereas the anhydrous form exhibits two peaks at 80-90 ppm corresponding to a carbon atom.

Additionally, the β-anomer of lactose exhibits a downfield shift of the C^{13} anomeric carbon peak when compared to the α-anomer. C^{13} NMR is a method that has to be conducted over a relatively longer time period than an H^1 NMR (~6 min) method and the former technique is less sensitive due to the lower relative abundance of C^{13} (1.1%) compared to H^1 (99%). On this basis, H^1 NMR has been used successfully to determine the anomeric content of lactose due to the occurrence of two separate doublets corresponding to the α- and β-anomers of lactose in the spectrum between 6-7 ppm. The ratio of the two anomeric forms of lactose in the sample can be calculated by using the integrated areas of the peaks (Altamimi et al., 2017; Jawad et al., 2014; Jawad et al., 2012). Some advantages of the NMR method include ease of preparation, non-ambiguous results and applicability to both amorphous and crystalline starting material.

Therefore, it is clear that H^1-NMR, a fast technique, can provide a clear interpretation of results with appropriate precision, rendering it a suitable method to analyse commercial lactose samples for anomer composition.

The aim of the study reported here was to evaluate and further optimize a H^1 NMR method for the determination of the variability in the anomeric content of commercially available lactose powders. DSC was used to initially characterize the lactose samples to investigate the potential shortcomings of analytical strategies that are based solely on measuring physical properties rather than true anomeric composition.

2 Materials and Methods

2.1 Materials

The lactose samples were stored in the laboratory under ambient temperatures. The information regarding their specifications is noted below. For particle size please refer to supplementary material. The pertinent information for the present study given by the suppliers has been summarized in

Table 2.

Table 2 Summary of supplier information of commercially available lactose powders

Sample name	**Supplier/Manufacturer & method of production.**	**Information from product sheets, specification and materials data safety sheet (particle sizing given in appendix A).**
α–Lactose Monohydrate	Sigma Aldrich: Method of production not given by supplier.	> 99% total lactose, < 4 % w/w beta lactose. Chemical structure given as the monohydrate. Chemical reagent.
Foremost 312	Foremost Farms / Kerry: Milled lactose.	Lactose monohydrate; Lactose 99% w/w; % Water 4.5 to 5.5; Used for wet granulation and capsule filling. Highly crystalline.
Tablettose 80	Meggle: Spray-agglomeration – water sprayed onto fluidized fine milled lactose particles.	Agglomerated alpha lactose monohydrate. Used for direct compression formulations and capsules.
Prismalac 40	Meggle: Coarse sieved alpha lactose monohydrate grades.	Mono-crystals of alpha-lactose monohydrate. Used for capsule filing.
Tablettose 70	Meggle: Spray-agglomeration – water sprayed onto fluidized fine milled lactose particles.	Agglomerated alpha lactose monohydrate, low fines content. Used for direct compression formulations, capsules and orally disintegrating tablets.
Tablettose 100	Meggle: Spray-agglomeration – water sprayed onto fluidized fine milled lactose particles.	Agglomerated alpha lactose monohydrate, higher fines content. Used for direct compression formulations, capsules and orally disintegrating tablets.
Ludipress	BASF A granulated mixture of lactose monohydrate 93% w/w, Povidone K30 and Cropovidone.	Lactose monohydrate is determined using a polarimetric assay. Used for direct

		compression tableting.
Granulac 140	Meggle: Milled alpha lactose monohydrate crystals.	Alpha lactose monohydrate. Narrow particle size distribution; cohesive particles. Used as a diluent in dry and wet granulation processing.
Granulac 230	Meggle: Milled alpha lactose monohydrate crystals.	Alpha lactose monohydrate. Narrow particle size distribution, cohesive particles. Used as a diluent in dry and wet granulation processing.
Sorbolac 400	Meggle: Milled alpha lactose monohydrate crystals.	Alpha lactose monohydrate. Narrow particle size distribution, cohesive particles. Used as a diluent in dry and wet granulation processing.
Starlac	Meggle: Co processed - 85 %w/w alpha-lactose-monohydrate and 15 %w/w white maize starch.	Used for direct compression formulations, capsules and orally disintegrating tablets. Spherical agglomerated particles with good flow properties.
Granulac 70	Meggle: Milled alpha lactose monohydrate crystals.	Narrow particle size distribution, cohesive particles. Used as a diluent in dry and wet granulation processing.
Flowlac 90	Meggle: Spray-dried suspension of fine milled alpha-lactose monohydrate crystals in a solution of lactose.	Spherical agglomerate shape, consisting of small alpha-lactose monohydrate crystals bound by amorphous lactose. Used for direct compression tablet formulations.

Combilac	Meggle: - Co processed 70 %w/w alpha-lactose monohydrate, 20 %w/w microcrystalline cellulose (MCC) and 10 %w/w white, native corn starch.	Used for direct compression formulations, dry granulation and orally disintegrating tablets. Spherical agglomerated particles with good flow properties.
Flowlac 100	Meggle: Spray-dried suspension of fine milled alpha-lactose monohydrate crystals in a solution of lactose.	Spherical agglomerate shape, consisting of small alpha-lactose monohydrate crystals bound by amorphous lactose. Used for direct compression tablet formulations.
Cellactose	Meggle: Co processed - 75 %w/w alpha-lactose-monohydrate and 25 %w/w powdered cellulose.	Used for direct compression formulations tablets. Spherical agglomerated particles.
Foremost 316	Foremost Farms / Kerry: Lactose monohydrate modified spray-dried.	A spray-dried mixture of crystalline and amorphous lactose Spherical particles; Lactose 99% w/w; % Water 4.5 to 5.5 Used for direct compression tablets and in capsule filling.
MCC 25	Foremost Farms / Kerry: Disintequick™ MCC 25 is a co-processed Lactose/MCC at a ratio of 75:25 %w/w Alpha lactose Monohydrate to MCC	Used for direct tableting. Need more info on the composition, properties and how much lactose is present with respect to the MCC.
β-lactose	Sigma Aldrich: Method of production not given by supplier.	> 99% total lactose, < or equal to 30 %w/w alpha lactose. Chemical reagent.

2.2 DSC method

A Q20 DSC apparatus attached to a cooler was used and a nitrogen gas flow of 50mL/min was maintained during the experiments. The DSC instrument was calibrated using 99.999% pure materials for temperature and enthalpy including indium, tin and lead that have a melting points of 156.6°C, 231.9 °C and 327.5 °C respectively (supplied by Perkin Elmer). Samples (4±2 mg) of lactose from each test batch were weighed accurately into aluminium DSC pans to the nearest 0.001 mg using a Sartorius balance. The pans were sealed with lids that had been pre-pierced to allow volatile material to escape during the heating procedure. Each sample was equilibrated at 25°C before heating the pan up to 250°C at 10°C/min. The sample was then set to equilibrate at 250°C, cooling to 25°C at 10°C/min. Sample pans were then re-weighed accurately (±0.001 mg) after analysis and the % weight loss calculated.

1.1 2.2 H^1 NMR

Dimethyl sulphoxide (DMSO, 0.7 mL) and 0.05% tetramethyl saline (TMS) (%v/v) (Goss Scientific Instruments, Crewe, UK) were used as a solvent and a signal reference for the experiment (at 0 ppm) respectively. Samples of β-lactose 4 mg (ACROS Organics, Leicestershire) or 6 mg α-lactose monohydrate (Sigma, Dorset) that had been stored at room temperature were dissolved in DMSO contained in 400 MHZ Wilmad NMR tubes (Sigma, Dorset, UK) under ambient conditions. Lactose samples were allowed to dissolve for 10 min before starting the analysis using a 400 MHZ Bruker Avance NMR. The length of the proton NMR analysis using a QNP probe was ≃10 min and 16 scans were carried out, employing a zg30 sequence (30° pulse applied prior to spectrum acquisition). This procedure was employed so that the analysis was carried out within 20 min thereby ensuring that minimal anomeric conversion occurred within the aprotic solvent, DMSO (Jawad et al., 2014; Jawad et al., 2012). However, before this procedure was finalised, the sample preparation method was investigated both with respect to the type of environment under which the NMR tubes were loaded and the mass of lactose used, as described in Sections 2.3 and 2.4.

1.2 2.3 Influence of sample mass

To investigate the role of sample weight on the precision of the NMR method (where the solubility of lactose in DMSO is reported previously to be ≥100 mg/mL (Keith and Walters, 1991). A predominantly α-lactose powder product (α-lactose monohydrate Sigma; > 96% w/w α-anomer) and a predominantly β-lactose powder product (β-lactose, Acros Organics; 20% w/w α-anomer) were analysed using 2.9 mg/mL, 5.7 mg/mL and 8.6 mg/mL (or 2, 4,

and 6 mg in 0.7 mL) DMSO. Samples were analysed within 20 min. The precision of the results was determined by comparing the standard deviation (SD) of the area of the integrated α-anomer peak with the constant solvent peak area.

1.3 2.4 H^1 NMR sample preparation under nitrogen

To investigate NMR preparation under nitrogen, NMR tubes containing lactose samples in DMSO were prepared inside a nitrogen glove bag (Glas-Col Inflatable glove chamber model X) after creating a flow of oxygen free nitrogen gas within the bag (approximately 5 min) and sealed with a gas tight seal. The NMR tubes, without caps, were placed in the glove bag with the measured DMSO aliquots. A positive pressure was maintained within the bag and after sample preparation, solutions of lactose in DMSO were analysed between 1-24 h after dissolution to determine the rate of change in the anomeric content of the lactose samples that had been prepared using this method.

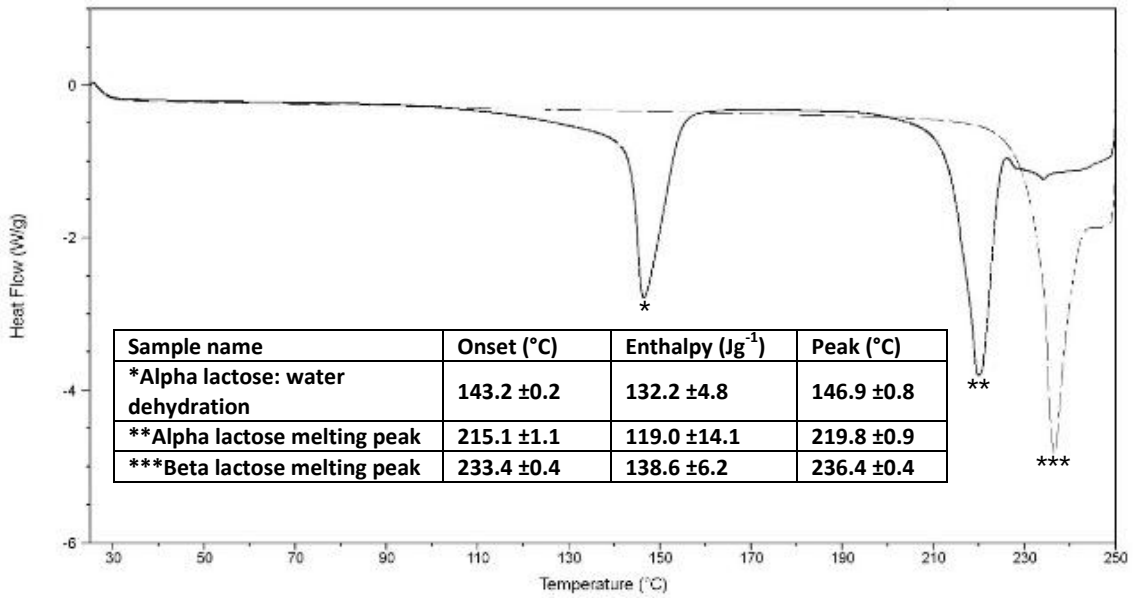
2.3 Powder X-ray diffraction (PXRD)

The crystallinity of the sample was investigated using a PXRD. The Rigaku MiniFlex 600 diffractometer (Rigaku, Tokyo, Japan) operated with Cu Kα radiation (1.5418 Å) at 40 kV and 15 mA, was used to collect patterns in the 2θ range from 3 to 40° at a speed of 2°/min and a step size of 0.01°.

3 Results

3.1 DSC analysis of commercially available lactose samples

A wide range of proprietary lactose samples were analysed. Figure 3 shows a representative DSC thermograph combining the results obtained from α-lactose powder (Sigma Aldrich, > 96 %w/w α lactose) with that obtained from β-lactose powder (ACROS Organics, ≤ 20%w/w α lactose).



Sample name	Onset (°C)	Enthalpy (Jg^{-1})	Peak (°C)
*Alpha lactose: water dehydration	143.2 ±0.2	132.2 ±4.8	146.9 ±0.8
**Alpha lactose melting peak	215.1 ±1.1	119.0 ±14.1	219.8 ±0.9
***Beta lactose melting peak	233.4 ±0.4	138.6 ±6.2	236.4 ±0.4

Figure 3 DSC analysis of α-lactose and β-lactose samples showing a representative thermogram with the integrated peaks for α-lactose monohydrate and β-lactose powder. n=6 for each sample ±SD.

α-lactose contains one hydrate molecule (H_2O) per lactose molecule. The water is accommodated within the matrix of the lactose crystal structure and is lost at ~ 143°C. As shown in Figure 3, the water loss peak integration range is 112.5 –162.5°C with the onset occurring at 143.2°C. The sharp nature of the peak indicates that the water is not residual content but is indeed the dehydration peak of α-lactose monohydrate (Chen et al., 2015). The lactose anomeric peak integration interval was set between 187.5 – 230°C (approximately) for α-lactose monohydrate and 212.5–250°C for β-lactose a sigmoidal peak integration was used for lactose due to the lowering of the baseline as a consequence of the degradation that occurs just after the melting point. The integration intervals were determined by considering the differences in the melting point of lactose anomers. This is apparent from Figure 3, where the onset of the melting peaks were 215.0°C and 233.5°C for α-lactose and β-lactose anomers respectively. The weight losses of α-lactose monohydrate and β-lactose powders (mean ± SD) were 5.0 ± 0.4 % w/w and 0.1 ± 0.1 % w/w respectively.

A melting reaction and degradation were observed for the α-lactose sample; whereas, only one melting peak was observed for the β-lactose sample.

Indium (the calibration material), being a stable metal produced DSC results that possessed greater precision in comparison to those generated by the lactose samples. When indium was subjected to DSC (n=6), the coefficient of variation (%CV) was 0.46 for the area of enthalpy. The onset temperature demonstrated a %CV of 0.03. The variance in the DSC results for the lactose samples were an order of magnitude higher than for the calibration material. This difference is reflective of the lower contact area of the lactose particles to the DSC pans, as these particles have a high surface area and void volumes within the powder bed.

DSC analysis of lactose reported in the literature has provided varied results. For example, the onset of the α-lactose melting peak (°C) has been variously reported as 195°C (Raemy and Schweizer, 1983), 215°C (Dudognon et al., 2006), 223°C using a different technique (Machado et al., 2000), 210 – 215°C (Kougoulos et al., 2010), 218°C (Kaialy and Nokhodchi, 2013) and 220°C (Kaialy et al., 2011b). The melting onset of α-lactose monohydrate in this study was 215°C and within the range previously reported. The enthalpy (J g^{-1}) of α-lactose monohydrate samples has been reported as 680 (Raemy and Schweizer, 1983), 134 (Drapier-Beche et al., 1999), 143 (Kaialy et al., 2011a) and 144 (Kaialy et al., 2011b). The value obtained in this study was lower (119 ±14 Jg^{-1}). The difference in these values can be attributed to the precise method of analysis, where the selection of the integration peak can be subjective. The melting temperature is a more precise measurement than the enthalpy when the study incorporates an indium standard. Repeatability (inter-day variation) of the melting temperature and enthalpy was 0.1% and 2.4% respectively (Toscani et al., 2012).

The variation in the melting onset and the enthalpies of the α-lactose melting and water loss of the lactose samples that were determined by DSC are detailed in

Table 3; the samples were analyzed as shown in Figure 3 DSC analysis of α-lactose and β-lactose samples showing a representative thermogram with the integrated peaks for α-lactose monohydrate and β-lactose powder.

Table 3 Monohydrate dehydration and α-lactose anomer melting enthalpy with respect to loading mass (n=6) obtained by integrating for water loss from 112.5–150°C and integrating the curve for α-lactose at 186.5– 205°C.

Sample name	α-lactose melting enthalpy (Jg^{-1})	%CV	Water dehydration enthalpy (Jg^{-1})	%CV	Melting onset °C	%CV
α–Lactose Monohydrate	119.0	14.3	132.2	3.9	214.8	0.4
Foremost 312	156.3	11.1	134.1	4.9	215.4	0.4
Tablettose 80	65.5	5.9	81.0	3.5	211.8	0.5
Prismalac	91.3	2.6	114.8	2.3	214.6	0.3
Tablettose 70	144.4	4.3	140.2	2.2	214.6	0.7
Tablettose 100	132.9	23.9	134.7	3.9	214.8	0.8
Ludipress	145.2	0.7	152.8	2.5	214.9	1.1
Granulac 140	142.0	2.1	139.9	2.4	214.1	0.2
Granulac 230	124.7	11.5	148.4	2.2	215.6	0.1
Sorbolac 400	122.7	6.8	149.4	5.4	214.0	0.2
Starlac	95.3	2.4	91.7	5.3	209.6	1.3
Granulac 70	119.3	5.0	125.0	4.9	211.8	0.2
Flowlac 90	119.2	5.8	141.6	5.5	212.1	0.1
Combilac	77.7	9.5	70.1	5.8	215.9	0.3
Flowlac 100	104.6	6.5	139.0	3.8	215.3	0.4
Cellactose	65.5	1.5	81.0	2.4	213.7	0.1
Foremost 316	165.1	1.7	143.5	0.8	213.6	0.0
MCC 25	98.2	8.1	68.0	2.9	209.8	0.5
β-lactose	N/A	N/A	N/A	N/A	233.4*	0.2

DSC is easy to use and is one of several techniques employed in the analysis of lactose (Table 1). The α-lactose in the samples is in the monohydrate form. It was expected that the melting enthalpy of the α-lactose would be directly proportional to the water loss enthalpy as the water of hydration is

removed from the α-lactose crystal. However, the results did not follow the expected trend (Table 3). It appears that there were other variables affecting the correlation between the melting peak and the water loss enthalpy. Such factors might include, the method of manufacture and/or the presence of other excipients in the lactose. For example, when the enthalpy change was expressed in Joules per gram of lactose (as opposed to Joules per gram of the sample loaded (

Table 3) there was still no statistical correlation between the α-lactose melting enthalpy and the water dehydration enthalpy.

3.2 NMR analysis of lactose

A sample of β-lactose powder (Sigma, Dorset, 80% β-lactose, 20% w/w α-lactose) was analyzed to confirm that the NMR analysis of lactose method was appropriate for differentiating between the two anomeric forms of lactose. The peaks corresponding to the protons of the α- and β-anomer of lactose occur in the 6–7 ppm region of the NMR spectrum (see Figure 4).

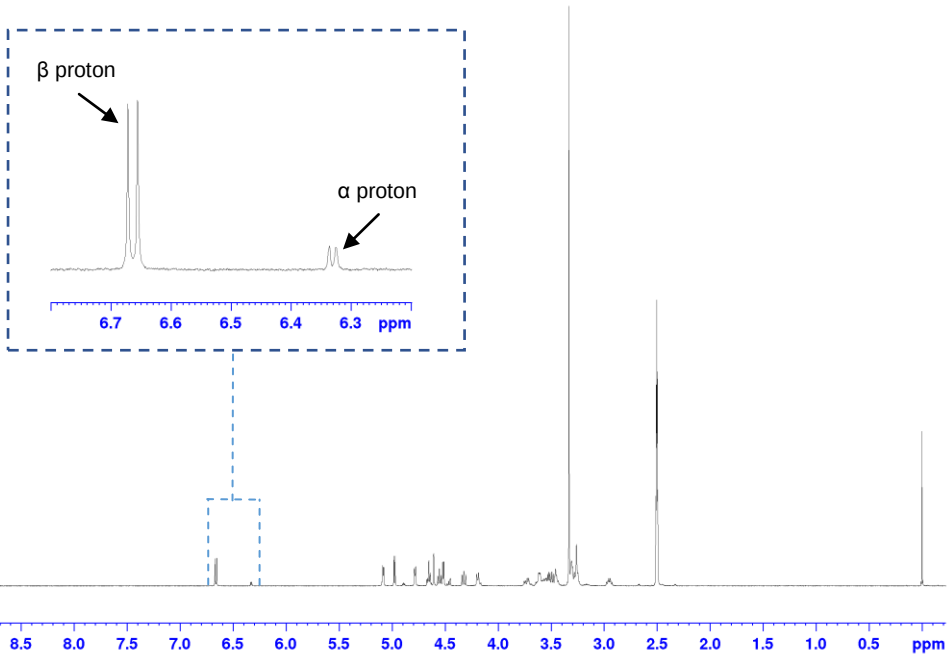


Figure 4 A representative NMR spectrum for a β-lactose powder sample with a magnified region showing the position of the α- and β-protons using a 400 MHz NMR.

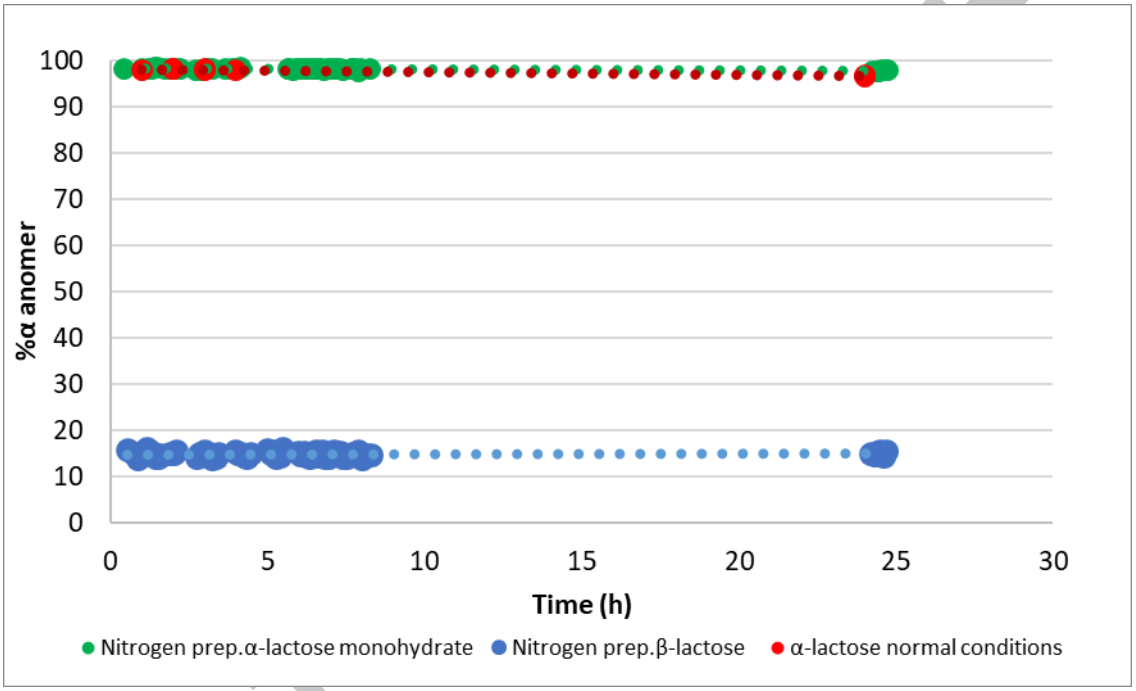
Due to the difference in orientation of a carbon in the anomeric forms of lactose, the peak of the proton from β-lactose occurs further downfield than the α-lactose and the ratio between the two peaks corresponds to the ratio of anomers in the sample.

3.3 Preparation of NMR samples under nitrogen gas

The analysis of lactose prepared under nitrogen was carried out to ensure that there was no significant change in the anomeric content of lactose when using this method of preparation (

Figure *5*).

Figure 5 Comparison between the change in anomeric content of lactose analyzed up to 24-h with and without preparation under nitrogen (6 NMR samples were used for each group to create the data points for the trendline).



The change in the anomeric content in the sample stored for 24 h was 4 times faster when not stored in nitrogen. If nitrogen was used the α-anomer content decreased from 98.2% to 97.8% w/w after 24 h, whereas in the absence of the gas the α-anomer content decreased from 98.0% to 96.3% w/w. The differences in the changes between 0 to 8 h were not statistically significant (t-test; $p>0.05$). Approximately 30 min after the solution was prepared, all the particles were dissolved and a true solution was formed. In the case of nitrogen-prepared samples, no significant change in the α- and β-ratios were detected over this initial 30 min preparation time. As can be seen in figure 4, the anomeric content remained constant from the first point up to 8 h.

3.4 Influence of loading mass on the precision of the NMR assay

Compared to many modern analytical techniques, solution based H^1 NMR has relatively poor sensitivity. NMR signal to noise ratio is influenced by many factors, but as the intensity

of the NMR peaks are, in part, proportional to the amount of analyte present within the magnetic field, the more concentrated the solution the better. For small molecules similar to the relative molecular mass of lactose, loading masses of 1 to 10 mg dissolved within the typical 0.7 mL of an NMR tube are common. However homogenous solutions are required, so it is important to avoid precipitation, as this dramatically increases the signal to noise ratio.

NMR has been used successfully to quantify the purity of organic samples when using a known internal standard as a reference whereas the DSC can be complicated by the complexity of the results (i.e. broad peak, irregular shaped peaks) and the material (i.e. particle-pan contact, head space and powder distribution) so that a concentration cannot be calculated (Mahajan and Singh, 2013). Quantitative NMR previously demonstrated high accuracy (99.9%) and precision (0.35% SD) when creating a calibration graph (Crouch and Russell, 2011). Such an application of NMR for the analysis of lactose samples has been shown to be precise with standard deviations in the 0.1% range (Jawad et al., 2012).

The H^1 NMR spectra can be affected by the amount of the lactose added to the 0.7mL of DMSO required for the experiment. The solubility of both lactose in DMSO is ≥100 mg/mL, which means that the lactose sample should fully dissolve in 0.7 mL; however, the time it takes to dissolve may vary as encountered during the experiment (Keith and Walters, 1991). The undissolved lactose decreases the sensitivity of the assay, which was determined by measuring the intensity of sample peak with reference to the solvent peak. If too much lactose is added in the DMSO the lactose takes longer to completely dissolve. This can cause the NMR run to take longer, due to greater background ‘noise’ cause by the few remaining undissolved particles. The results may also become inadequate if there is too much sample because the undissolved lactose generates a non-homogeneous of the sample and this in turn decreases the sensitivity of the instrument. Samples of lactose prepared using nitrogen gas are not affected since the samples were allowed to completely dissolve before running the experiment. Thus, sample mass optimisation was determined without the use of a nitrogen environment to investigate further the use of less-controlled assay conditions. Sample sizes of 2, 4 and 6 mg of α-lactose monohydrate were employed (Figure 6 and the amount of undissolved α-lactose particles increased as a function of increasing weight but decreased as a function of time. A sample of β-lactose was also analyzed similarly and the results are shown in Figure 7 .

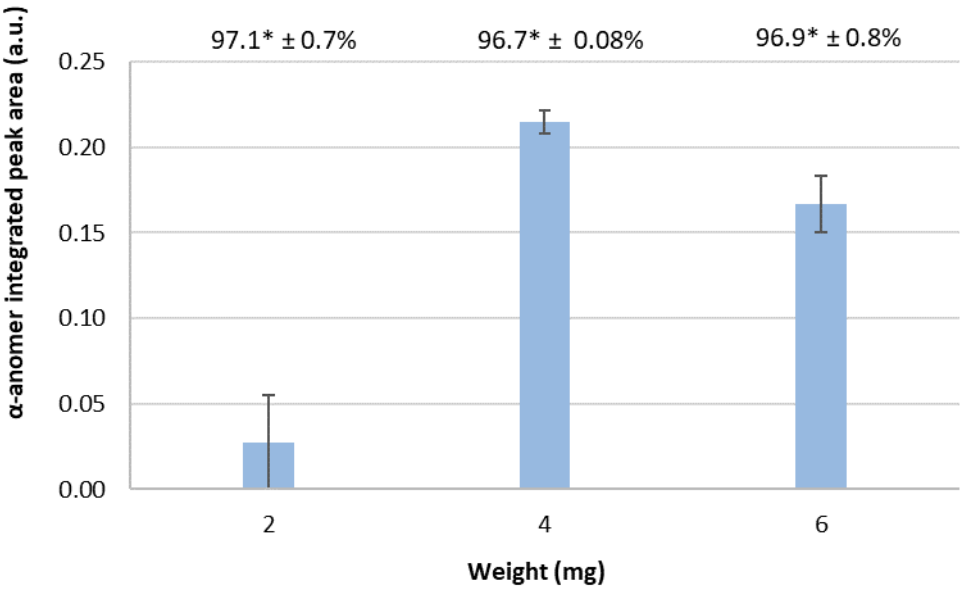
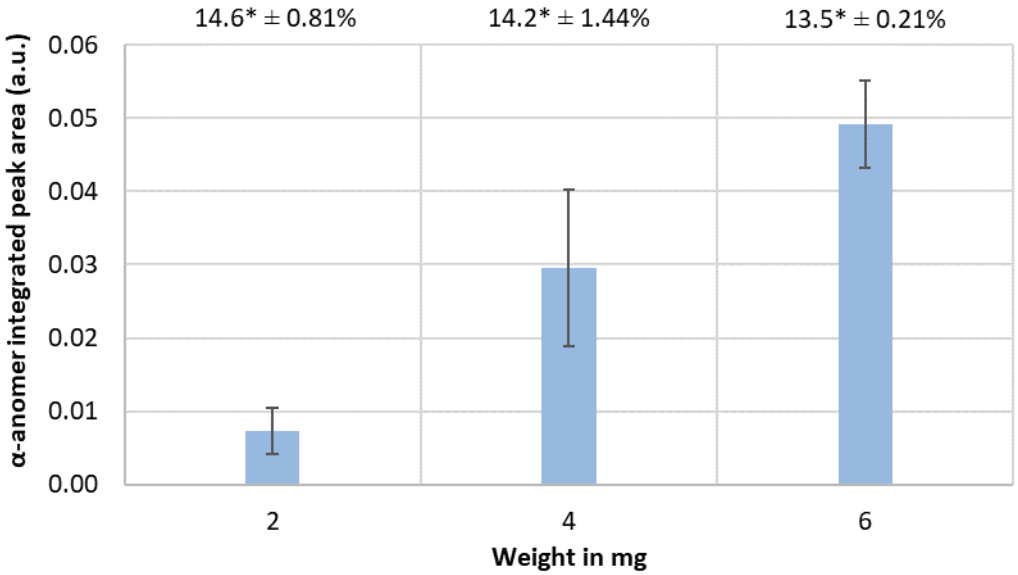


*Figure 6 Sample loading weight for NMR analysis of α-lactose (MEGGLE Granulac 240) samples (n=3). The ratio of the α-lactose peak intensity with reference to the solvent (DMSO) peak of predominantly α-lactose powder is shown. * α-lactose content ± standard deviation. Error bars = SD.*

The anomeric content for the 2, 4 and 6 mg sample sizes was determined to be 97.1% ±0.7, 96.7% ±0.075, and 96.9% ±0.08 w/w α-lactose respectively. The results of the analysis of the 4 mg samples displayed the lowest standard deviation and the largest peak area

Figure 6). Therefore, 4 mg in 0.7 mL of DMSO was chosen to be the recommended



optimum concentration when analyzing content lactose samples with high α-anomer content.

*Figure 7 Sample loading weight for NMR analysis of β-lactose (ACROS ORGANICS) samples (n=6). The ratio of the α-lactose peak intensity with reference to the solvent (DMSO) peak of predominantly β-lactose powder is shown. * α-lactose content ± standard deviation. Error bars = standard deviation.*

The anomeric content of the assayed β-lactose powder was calculated as 14.6% ± 0.81, 14.2% ± 1.44, and 13.5% ± 0.21 w/w α-lactose for the 2, 4 and 6 mg samples respectively. Therefore, as the 6 mg samples displayed the lowest standard deviation and the highest peak area, samples made using this concentration were considered to provide the highest precision. An accurate estimate of α-lactose monohydrate, can be obtained using a lower sample size when β-lactose is present as an 'impurity' in comparison to the case where β-lactose is the principal component. For an unknown sample, it is recommended to use 6 mg as this would lead to a maximum standard deviation of 0.8% anomeric content. Should a sample be suspected to contain high alpha content, an additional run with 4 mg is recommended.

3.5 Library of lactose samples analyzed by NMR

A library of lactose samples was characterized using the NMR analysis method described previously (Table 4). Using the results obtained from the sample weight investigation, the sample masses of predominantly α- and β-lactose samples that were employed in the studies were 4 and 6 mg respectively.

Table 4 *Library of the anomeric content of commercially available lactose samples analyzed by H1 NMR n=6* [a]*The %CV associated with the β-anomer content was the same as that of the α-anomer content.* [b]*microcrystalline cellulose/lactose mixture.*

Sample	% α-anomer	%CV	% α-anomer (manufacturer)	% β-anomer[a]
α-Lactose Monohydrate	98.4	0.14	≥96 %	1.6
Foremost 312	98.4	0.07	N/A	1.6
Tablettose 80	98.1	0.28	N/A	1.9
Prismalac	97.9	0.11	N/A	2.1
Tablettose 70	97.8	0.14	N/A	2.2
Tablettose 100	97.8	0.15	N/A	2.2
Ludipress	97.8	0.12	N/A	2.2
Granulac 140	97.7	0.20	N/A	2.3
Granulac 230	97.4	0.07	N/A	2.6
Sorbolac 400	96.9	0.14	N/A	3.1
Starlac	96.9	0.08	N/A	3.1
Granulac 70	96.9	0.21	N/A	3.1
Flowlac 90	96.1	0.16	N/A	3.9
Combilac	95.6	0.06	N/A	4.4
Flowlac 100	95.0	0.12	N/A	5.0
Cellactose[b]	94.6	2.18	N/A	5.4
Foremost 316	94.1	0.75	N/A	5.9
MCC 25	84.6	0.64	N/A	15.4
β-lactose	16.4	1.27	~20	83.6

The method of production of the lactose products can be found in Table 2. In summary, the milled and sieved samples generally possessed a higher α-lactose content than the co-processed samples where the α-anomer content relative to β-anomer content was as low as

84.6%w/w for MCC 25. The samples that were spray-dried exhibited a wide range of anomeric content, with the lowest α-lactose content produced by this method being 94.1% w/w (i.e. Foremost 316). Some manufacturers did not state the method of production and others seemed to use the term α-lactose monohydrate to suggest that the material was 100% w/w α-lactose. The sample with the highest %CV (2.18%) was found to be the co-processed material, Cellactose, which is a mixture of 75:25 % w/w α-lactose monohydrate and cellulose.

3.6 Comparison between DSC and NMR analytical techniques

The data indicated that the enthalpy of water loss obtained by DSC does not show a consistent correlation with the α-lactose content determined by NMR ($y = 7.6088x - 613.9$ and $R^2 = 0.0992$). DSC has been used as a quantitative tool for sieved samples of lactose (Kaialy et al., 2012), but in this study non-sieved samples were employed. Samples were not sieved, since the commercially available lactose samples are sold to users that are searching for bespoke material that should not require further preparation. Additionally, some of the lactose products that were analyzed in the current study did contain other excipients where the derived enthalpies were expressed as J g^{-1} of material rather than J g^{-1} of lactose. When these samples were removed from the data set, still no statistical correlation was observed. There was also a poor correlation between the melting peak enthalpy and anomeric content of the lactose samples ($y = 11.756x - 1024.1$; $R^2 = 0.1991$). The mean %CV was also used to compare the precision of each method. The %CV of the data from the samples assayed by NMR (0.53) and DSC (6.88) showed that they are very different and NMR is more than ten times more precise. Samples of β-lactose were initially included in the analysis, but there were no samples that contained 30% – 85% w/w α-lactose; therefore, the samples plotted include the predominantly α-lactose powders.

3.7 PXRD of lactose samples

In order to investigate the differences in the anomeric content of some of the lactose library samples, PXRD analysis was carried out on samples of lactose that were prepared using different methods by the manufacturers (Table 2). A sample of spray-dried, milled, and spray agglomeration were analyzed by PXRD. Additionally, α-lactose monohydrate (crystalline) and β-lactose (anhydrous; Sigma) were also analyzed by PXRD (Figure 8).

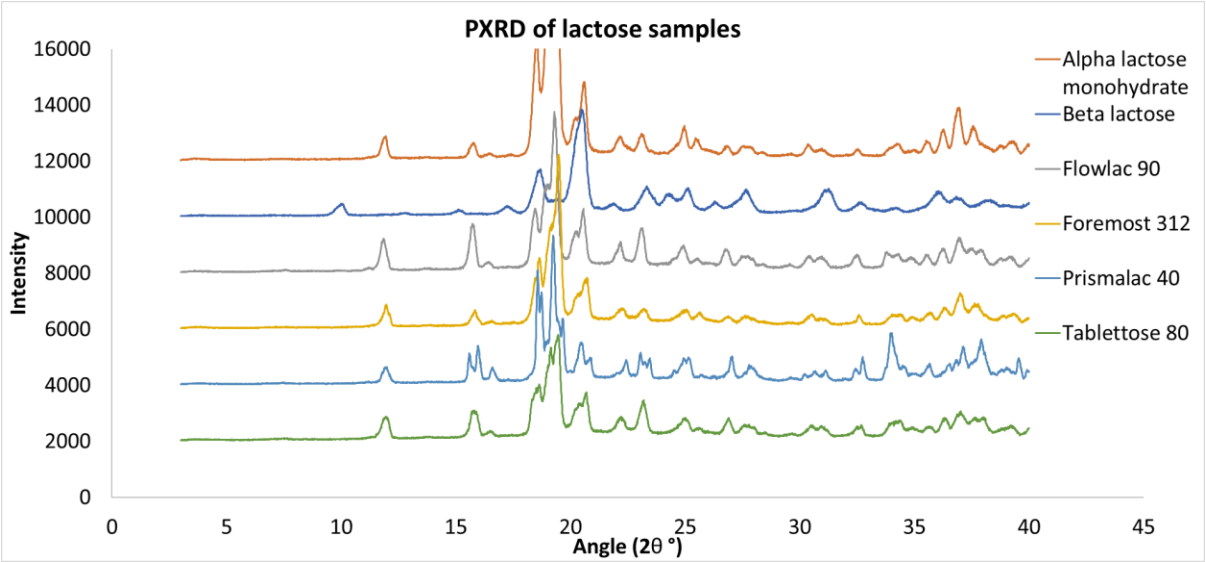


Figure 8 Overlay of PXRD spectra of lactose samples

There was a variation in the intensity of the peaks between the samples. However, all the samples exhibit profiles with clearly defined 'sharp' peaks, indicating that the samples are mostly crystalline.

4 Discussion

4.1 Pharmaceutical lactose production

The manufacturing process used to produce lactose powder determines the anomeric content of the marketed excipient (Appendix). There are several major manufacturers of lactose worldwide, such as those based in the USA (Kerry; Foremost), Netherlands (Borculo), Germany (Meggle) and New Zealand (Fonterra) (Rowe et al., 2006). Lactose products that are predominantly crystalline α-lactose monohydrate are prepared using different milling and sieving conditions that result in different flowability profiles, particle size distributions and bulk densities (Nickerson and Moore, 1974).

Crystalline α-lactose monohydrate possesses a tomahawk shape whereas β-lactose has a needle-like shape and may look irregular if the sample is crushed into smaller particle during manufacturing processes. Such anomer specific physical differences are likely to affect the anomeric content of the final grade when separating the lactose powder by sieving. During the production of crystalline lactose powders, the sugar is affected by many external parameters that will influence the anomeric content of the final product. For example, water activity, temperature, pH and the time taken for unit processes such as crystallization, drying and milling will influence the amount of α-lactose and β-lactose observed (Jawad et al., 2012; Lefort et al., 2006).

Spray-dried and amorphous lactose products are also available for direct compression to form tablets (Meggle, 2018). For predominately amorphous products, the spray-drying method requires the lactose to be in solution before the atomization of the solvent in a hot environment (usually 100 – 200°C). The fast nature of heat exposure (a few seconds) does not allow lactose to crystallize, as the solvent evaporates leaving the amorphous lactose product as the residue to be collected. However, for the spray-dried lactose powders featuring in table 2 and investigated in this paper, a suspension of alpha lactose monohydrate crystals in a lactose solution was used as the feed solution into the spray-dryer rather than solely a solution of lactose. Preparative milling was used to reduce the particle size of the suspended particles to allow flow through the drying atomizer. The manufacturer's choice of spray-drying is to achieve optimum compressibility and a targeted particle size range, but very little has been published on the anomeric distribution of these materials. When atomized the droplets of suspension will contain several of the milled crystalline α-lactose monohydrate particles, thus it is expected that the resulting powder will be highly crystalline. However, the disperse lactose solution surrounding the particles will

undoubtedly form an amorphous layer on the surface of these crystals when the water is removed in the drying chamber. As the water quickly evaporates, the suspended particles within the droplets are forced together leading to an agglomerated particle, with the individual fine crystals held together by the amorphous surface layer. The anomeric composition of this amorphous surface 'glue' is unknown, but as it formed from an aqueous solution some beta amorphous lactose will be present. Upon collection and storage, it would appear, from both the PXRD results and the DSC measurements presented here, that significant concentrations of amorphous lactose do not persist into the final powder. This is either a result of the amorphous layer contributing only a small undetectable fraction or the amorphous material re-crystallizing over time.

Flowlac 100 and Flowlac 90 are prepared by the spray-drying of a fine crystal suspension (Meggle, 2018). DSC and PXRD, Table 3 and Figure 8, indicate the highly crystalline nature of Flowlac powders. These grades have lower amounts of α-lactose, 95 & 96% w/w respectively, compared to samples prepared using other methods, where α-lactose is more predominant (Table 4). The residual β-lactose present, as discussed above, will have originated from the aqueous lactose solution surrounding the suspended crystalline α-lactose monohydrate particles in the original feed suspension.

The Flowlac 100 lactose has been reported to show a small re-crystallization peak as determined by DSC, when the samples were sieved prior to assay (Kaialy et al., 2012). In this study, the re-crystallization peaks were almost non-existent, indicating the low presence of amorphous content in the sample, support also by the PXRD data presented in Figure 8. Therefore, it is possible that the smaller particle size fraction of this grade of lactose might have an observable amorphicity than the sample as supplied (i.e. no preliminary sieving). Should any amorphous regions be located on the surface of the lactose this would have implications for potential differences in the adhesion of active pharmaceutical ingredient (API) to different sized fractions of lactose when used as carrier in DPIs. Such amorphicity, because of the higher surface energy in comparison to crystalline lactose may adhere to some of the API more tenaciously, resulting in more of the latter being deposited higher in the respiratory tract with the greater-sized lactose.

Batches of pharmaceutical grade lactose are now tailor-made so as to generate the desired properties for use in different applications; such as carrier particles in DPIs, fillers in capsules and diluents in tablets. The different grades of lactose are routinely distinguished by the manufacturer in terms of particle size (distribution); however, the anomeric content as a grading is rarely specified. The results reported here show this can vary and may be a

factor that should be reported in future quality specifications. Finally, the lactose products can be mixed with the other components of the medicine (i.e. API and other excipients), using one of the three main blending methods such as dry mixing followed perhaps by wet granulation. The latter involves further exposure to small amounts of solvents and potentially a humid atmosphere which might subsequently affect the anomer content further. Other pharmaceutical processes such as milling, spheronisation, freeze drying, spray-drying etc. will also influence the composition of final product (Jawad et al., 2014; Lefort et al., 2006). Accordingly, the original isomer composition of each lactose grades, as determined and given in Table 4, is likely to change during processing.

4.2 Stability of lactose and quality assurance

The stability of certain lactose products has been brought to question by Altamimi et al. 2017. Lactose production methods, storage and the utilization in medicines can cause changes to the anomeric content of the product that is attributable to physical and environmental stress such as milling and spray-drying (Willart et al., 2004) and change in humidity. When purchasing lactose samples, the specifications that are provided will include limits on water loss-on-drying, particle size distribution and the composition of mixture products (lactose and MCC). The properties of the product need to be specified with a high level of certainty to be acceptable for use by consumers and manufacturers. To date, the anomeric content of the lactose material is generally not well characterized and hence not always quoted, although some product specifications do indicate an approximate and sometimes a minimum % anomeric content to be present in the sample.

For example, the aerosol performance from carrier-containing DPIs is highly dependent upon lactose composition (Traini et al., 2008). The complex nature of commercially lactose grades, being dependent on the methods of isolation or preparation results in some of the data previously reported in the scientific literature as being difficult to interpret. The significance of amorphous versus crystalline content (Vromans et al., 1987; Rassu et al. 2006; Omar et al. 2015) for example maybe placed ahead of anomer composition as being the determinant factor in tableting properties, despite the latter content varying dependent upon the preparations conditions of the lactose. Nevertheless anomer composition has been reported previously to influence the consolidation and compaction of tablets (Bolhuis et al. 1985; Riepma et al. 1990; Riepma et al.1992; Bolhuis and Zuurman 1995), crushing strength (Vromans et al. 1985) and disintegration and dissolution (van Kamp et al. 1986). There are well-established links between these pharmacopeia parameters and the performance of a tablet upon administration therefore; changes in the anomeric content will alter the efficacy of tablets and most likely lactose containing medicines in general. Furthermore, since β-

lactose is markedly more compactable than α-lactose samples (Ilic et al., 2009), this will ultimately affect the friability and hardness of tablets that need to pass specific standards set by the manufacturer. Stability tests are also carried out before these tests which involve the exposure of samples to temperatures of up to 40°C and 75% RH. Such conditions can catalyse changes in anomeric content and result in altered tablet properties (ICH, 2003).

4.3 Lactose analysis methods

Traditionally employed lactose analysis techniques do not routinely consider anomeric content, such as HPLC and enzymatic quantification assays, which have been used to quantify lactose in solutions (i.e. milk). Recent studies have shown that the former methods are not as adequate as LC-MS to quantify lactose, which proved to have a higher level of repeatability and precision (Trani et al., 2017). DSC has also been used widely to analyze lactose and is an easy tool that determines the presence of crystalline lactose. The melting curve of the lactose anomers can be defined using the technique (ca. 210+°C), but since the material degrades at temperatures soon after this, then it is not sufficiently sensitive to carry out accurate anomer quantification (Figure 2). Therefore, although DSC can be used for identification of the presence of an anomer, quantification by analysis of the melting peak is not feasible (Ilic et al., 2009). Both predominantly α-lactose and β-lactose powders have been investigated extensively by DSC in the literature and other studies investigate some grades of lactose (Kaialy et al., 2012); however, none of the previous studies have carried out a comprehensive analysis of all grades using DSC.

An advantage of DSC analysis is that it can determine the amorphous content of a lactose sample but its sensitivity to do this is again limited by the fact that it requires at least 20% w/w amorphous content for the quantification of crystallinity to be meaningful (GombÁs et al., 2002). If analysts require a qualitative measurement, DSC may be adequate; however, for a full quantitative assessment, NMR can be used as it has demonstrated a real-time determination of anomeric content. When analyzing DSC curves, there is a certain subjectivity involved in determining the exact onset and end of the peak integration. In addition, the origin of the wide variance in the α lactose melting enthalpy will undoubtedly have a contribution from mutarotation occurring in the lactose sample present in the DSC pan during the heating cycle. Water vapour liberated by the temperature driven dehydration will induce inter-conversion between the α and β-lactose anomers as the vapour passes through the powder bed before it leaves the DSC pan. It is well known that β-lactose is more stable above 90’C in saturated aqueous solutions of lactose, thus an increase in the amount of this anomer is expected because dehydration begins at approximately 140’C. The enthalpy contribution of mutarotation will be subsumed into the endothermic peak associated

with dehydration, which has a broad temperature range. The extent of any mutarotation and thus alteration in the anomer ratio in the heated lactose samples will depend on many factors, for example it will depend on the starting anomeric content, amount of monohydrate present, particle size, loading mass, headspace and the porosity of the particles. All of these parameters varied between each type of lactose tested, and so this was reflected in the range of enthalpies for both dehydration and melting given in Table 3. The aim of the work reported here was to determine the variability in the anomeric content of commercially available lactose powders. It would appear that because of many contributing factors, DSC was unable to provide quantitative measurements of the anomeric composition, whereas the NMR method was able to. The variance of the data presented in Tables 3 & 4 supports this conclusion as the mean %CV of the samples analyzed by DSC is five times greater than the mean %CV of the results obtained using the NMR technique. The performance of the NMR can be attributed to the unambiguous and separate NMR signals for α- and β-lactose as a consequence of the difference in molecular structure.

‘The lack of a correlation between the size of the DSC melting endotherms and the NMR results may have also been due to the presence of amorphous content within the samples (Table 3). PXRD was carried out on representative samples to explore this potential contributing factor. Although the results showed that there was some variation in the crystallinity of the lactose samples, an overlay of the PXRD data showed that the samples comprised mostly crystalline material. Accordingly amorphous content is not the main contributing factor to the poor correlation between the results obtained from the thermal and the NMR methods of analysis. Thus, as the temperature of a sample increases during DSC analysis, a change in the properties of the crystalline lactose samples occurs which is mostly related to the impact of dehydration and particle properties. Therefore, the melting curves would not be directly proportional to the anomeric content found in the unambiguous NMR spectra of a lactose sample that has not been heated to the same temperatures as the DSC sample.

The results of the PXRD experiment did indicate some degree of variation in the peak patterns and thus the crystallinity of the samples. The issue with employing PXRD to determine the crystallinity of lactose is that there is always a part of the sample that is not α-lactose monohydrate that can contribute to the intensity of some peaks. For example, there is no clear PXRD intensity peak pattern that represents only one lactose anomer because there is always an impurity of the other anomer in the sample. In this study it has been

shown that the samples that are declared as being α-lactose monohydrate do indeed contain some β-lactose by the NMR method. There may be a contribution from the β-lactose to the peak being used as a reference. Such impurities can make it difficult to accurately quantify the precise % crystallinity of the sample. These observations further support the use of NMR in the unambiguous determination of the anomeric composition of lactose samples.'

There may be some potential in employing a hyper DSC method to overcome some of the challenges encountered when analyzing lactose powder. For example, a high heating rate may allow the users to detect the melting point while not allowing the samples sufficient time to degrade (Saunders et al., 2004). Other parameters that can affect the results obtained when using this technique include the influence of particle size and conduction of the material. Better control of water content and the amorphous/crystalline ratio may improve the correlation between DSC data and the samples' anomeric content, but the benefit of the NMR data is that they are unambiguous. This is supported by the fact that the 'uncontrolled' NMR analysis provided close values to the NMR analysis of samples prepared in a 'controlled' nitrogen environment.

By analysing a library of samples from the market, it is apparent that there is variability in the anomer content of different lactose grades from different suppliers (Table 4). The main limitation of the NMR technique described in the literature is the time-sensitivity of the method (Altamimi et al., 2017; Jawad et al., 2014; Jawad et al., 2012). By preparing samples under nitrogen, it was demonstrated that the NMR analysis method became less constrained by time, allowing more freedom when running NMR samples while retaining the integrity of the analysis. This novel approach ensured a very accurate representation of the anomeric content can be established in a number of samples within one working day.

4.4 NMR method evaluation

The NMR analysis method of lactose was proven to be simple and fast with an actual analysis time of 6 min. Typically in analytical laboratories, NMR samples are loaded and analyzed in a queuing/automated fashion, thus it may sometimes be difficult to restrict sample waiting times to <20 min. In an attempt to obviate the 20-min restriction on time between preparation in DMSO and complete analysis, an alternative preparation method using a nitrogen glove bag connected to oxygen free nitrogen gas was developed. By using a glove bag to contain a nitrogen atmosphere, many samples can be prepared with relative ease. In samples that are prepared under nitrogen, the particles were completely dissolved in solution and this enabled an NMR spectrum to be gained with lower background noise than samples that were run quickly without nitrogen.

After 24 h, a decrease of approximately 0.5% w/w α-lactose was found when using nitrogen, whereas in the absence of a nitrogen environment, a change of 1.7% w/w was detected after 24 h. These data show that mutarotation does occur at a relatively slow rate and thus depending upon the accuracy required, it may be acceptable for users to assay the samples without the use of nitrogen in the same day if it is acknowledged that an approximately 2% error in anomer content is acceptable.

4.5 Conclusion and recommendations

Lactose manufacturers employ several techniques to provide the specifications of lactose products detailed in pharmacopoeias (Kellam, 2008). For example, In the US Pharmacopoeia, anhydrous lactose products that are labelled with a specific purity of anomeric content of lactose must, by definition, test the anomeric content of the samples. In this case a GC method is advocated where the prescribed method requires several mixing and tube transfer steps to produce a 5 µL aliquot for GC injection. Additionally, in the pharmacopoeia, the anomeric content is not required to be assessed for the monohydrate form of α-lactose. According to the handbook of pharmaceutical excipients the 'isomer' ratio of anhydrous lactose powder is not a mandatory test in the European Pharmacopeia but is mandatory in the US and the Japanese Pharmacopoeias (Rowe et al., 2006), although there is variation between Pharmacopoeia monographs on how to deal with the anomeric specification of lactose (α anhydrous) and if it is a requirement. As for the monohydrate and spray-dried forms, there is no requirement to determine the anomeric content in the pharmacopoeias.

The results from this study show that there is a variability in the anomeric content in lactose products. For the labelled β-lactose sample, the actual value was 5% w/w different from the label claim and for samples that were declared to be α-lactose, up to 6% w/w β-anomer could be measured in the sample. Several of the other samples did not provided an anomeric content value in their specifications. With the revised technique described in this study, we would submit that its application for new lactose-containing products be considered since there is an emerging opinion in the literature that the anomeric content of lactose could play an important role in a medicine's pharmaceutical performance. For example, a study has shown that the anomeric content was a factor in determining the efficiency of carrier properties of lactose when employed in DPIs (Kaialy et al., 2011a).

We suggest a H^1 NMR method for the monitoring and surveying of lactose samples in a quick and efficient manner. Preparation in nitrogen should be used when samples are left for

longer than 20 min before analysis. In time, with further development such a method could form the basis of a sound monograph requirement.

Supplementary materials

Supplier/grade Typical	Particle size distribution (%)												%α anomer
	<32µm	<45µm	<63µm	<75µm	<100µm	<150µm	<200µm	<250µm	<315µm	<400µm	<600µm	<800µm	
Foremost Farms USA													
Foremost Lactose 312		64 - 80			94-100								98.4
Foremost Lactose 316					30-55			2					94.1
Meggle GmbH													
GranuLac 70					50					99.5			96.9
GranuLac 140	30				90	100							97.7
GranuLac 230	75		96		99.5								97.4
PrismaLac 40							4					100	97.9
SorboLac 400	95		99.5										96.9
Tablettose 100			12		22	42		77		98			97.8
Tablettose 80			20							85		100	98.1
Tablettose 70			1			25				90			97.8
Flowlac 90			10			50		90					96.1
Flowlac 100			10			50		90					95.0
Combilac			10				50		90				95.6
Ludipress			up to 15				40 - 60			>90			97.8
Starlac	10					50		90					96.9
Cellactose		10					50		90				94.6
Kerry													
MCC 25													84.6
Sigma													
Alpha lactose monohydrate						--							98.4
Acros Organics													
Beta lactose						--							16.4

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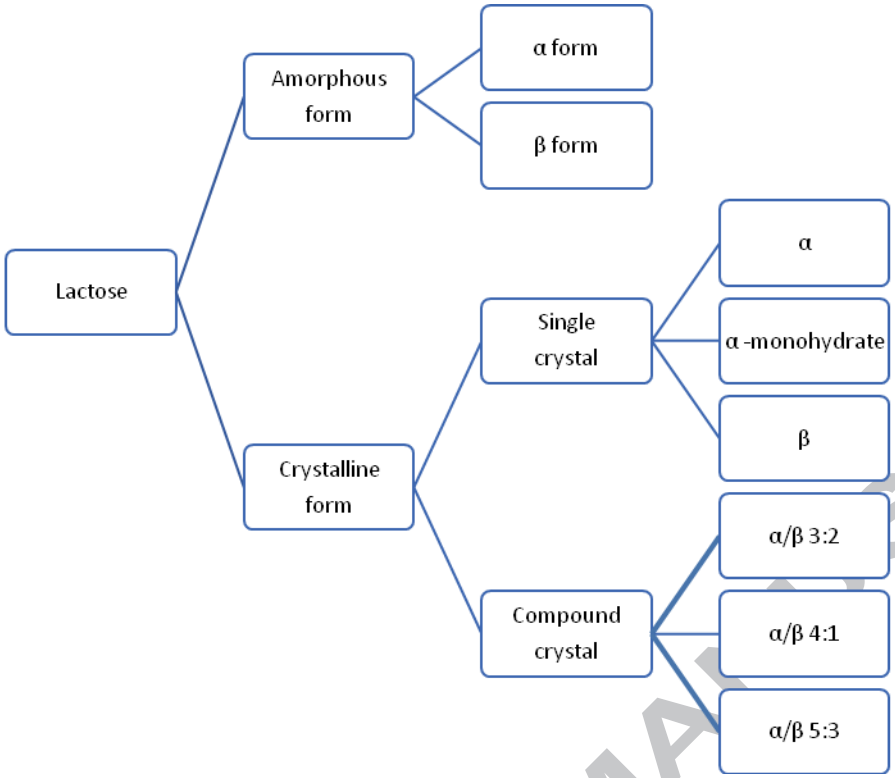
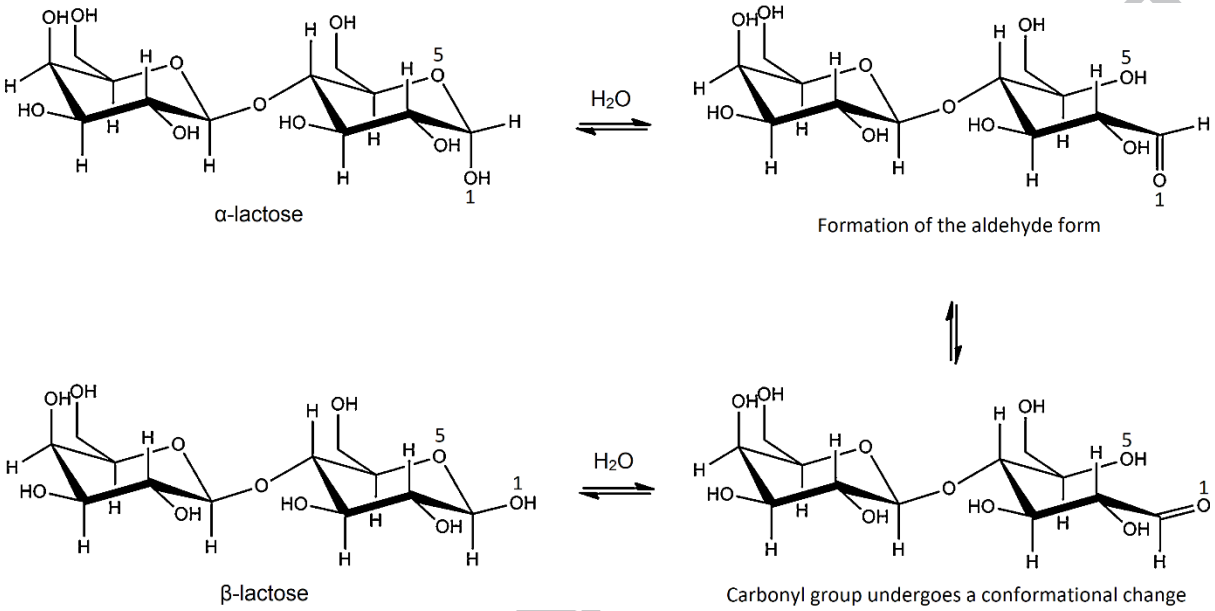


Figure 1 The solid forms of lactose available for manufacture (adapted from Jawad et al., 2015 with permission)

Figure 2 The forms of lactose that exist during mutarotation



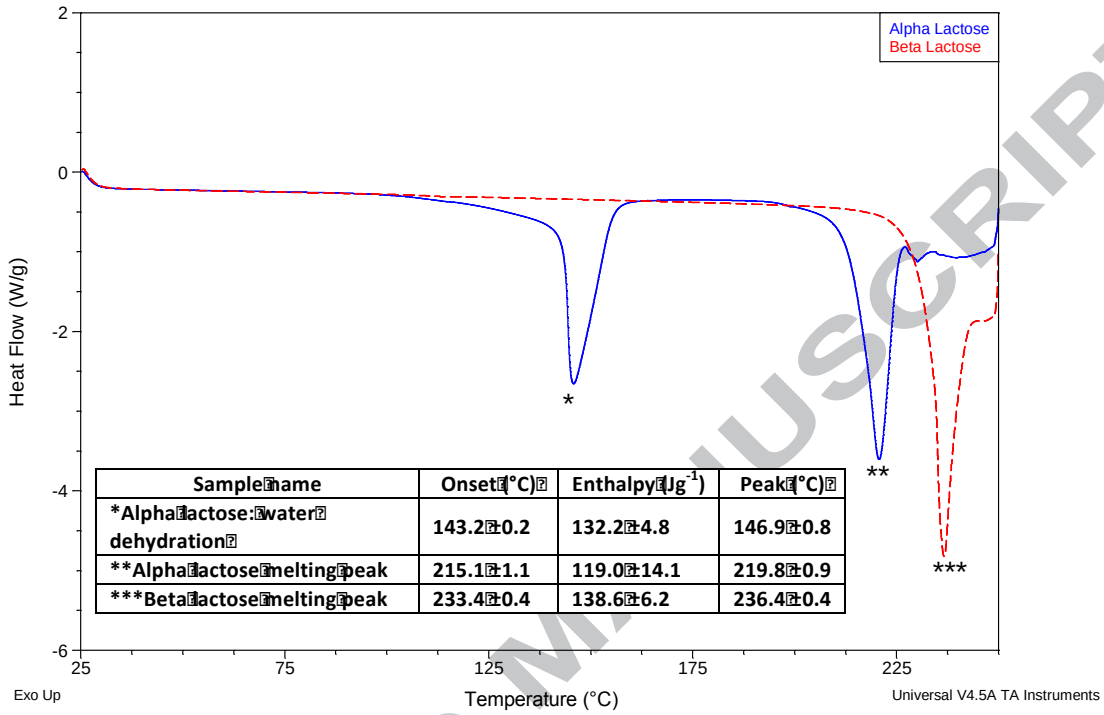


Figure 3 DSC analysis of α-lactose and β-lactose samples showing a representative thermogram with the integrated peaks for α-lactose monohydrate and β-lactose powder, n=6 for each sample ±SD.

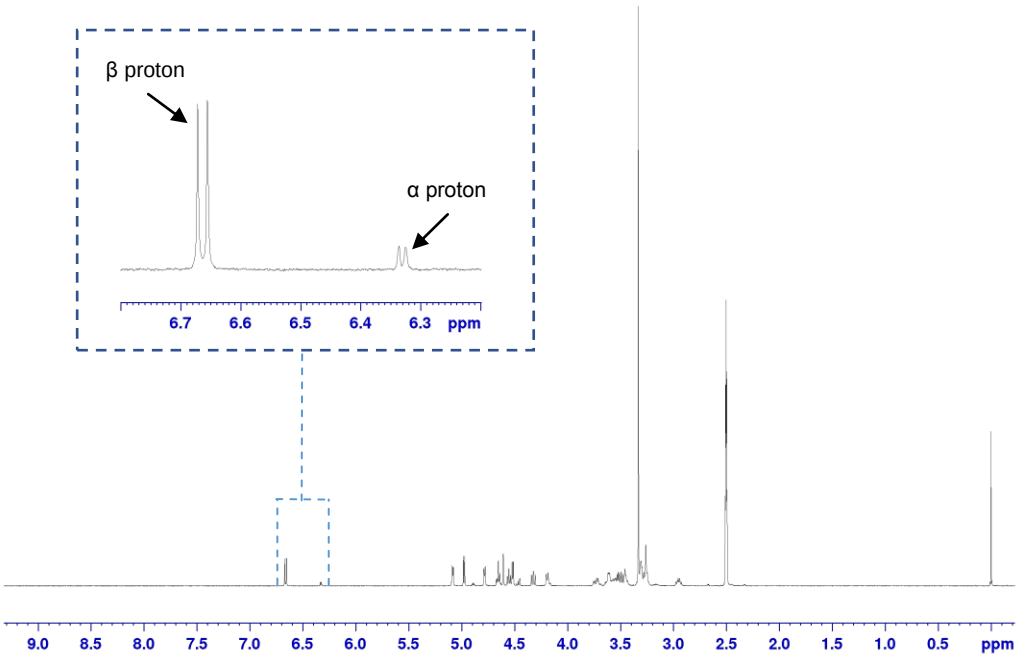


Figure 4 A representative NMR spectrum for a β-lactose powder sample with a magnified region showing the position of the α- and β-protons using a 400 MHz NMR.

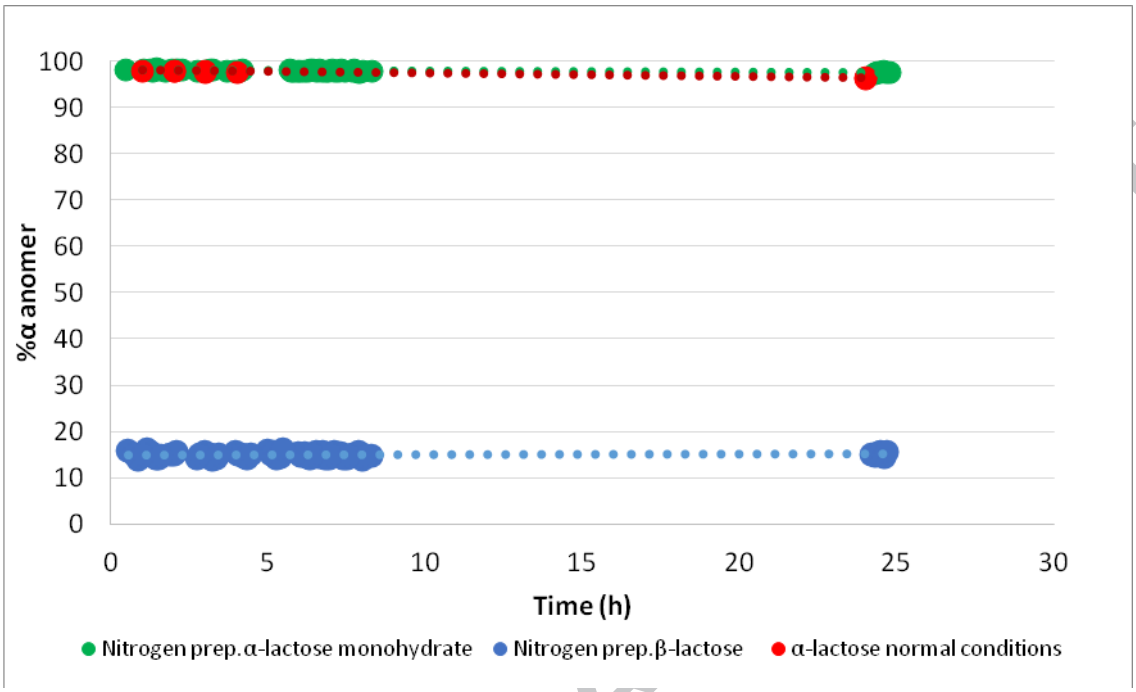


Figure 5 Comparison between the change in anomeric content of lactose analyzed up to 24h with and without preparation under nitrogen (6 NMR samples were used for each group to create the data points for the trendline).

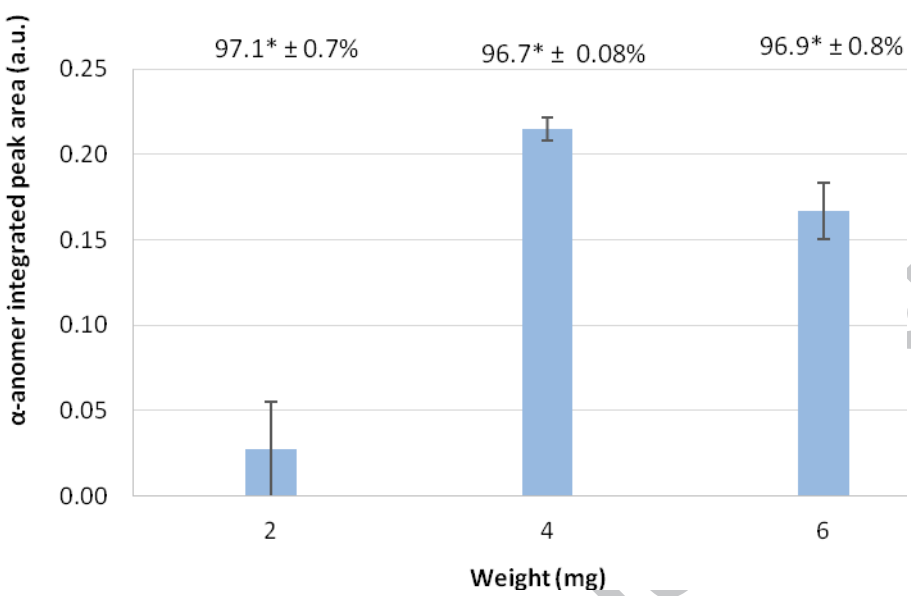


*Figure 6 Sample loading weight for NMR analysis of α-lactose (MEGGLE Granulac 240) samples (n=3). The ratio of the α-lactose peak intensity with reference to the solvent (DMSO) peak of predominantly α-lactose powder is shown. * α-lactose content ± standard deviation. Error bars = SD.*

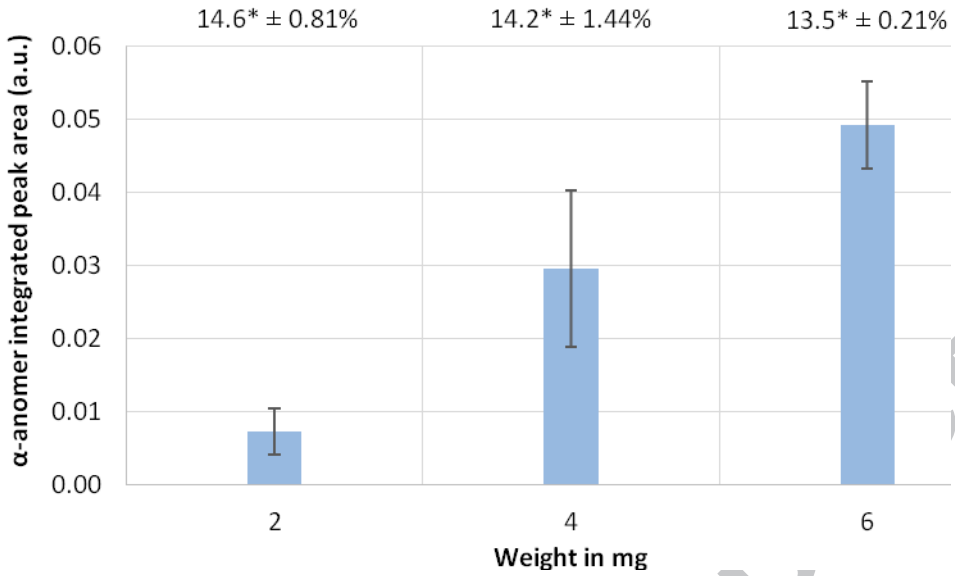


*Figure 7 Sample loading weight for NMR analysis of β-lactose (ACROS ORGANICS) samples (n=6). The ratio of the α-lactose peak intensity with reference to the solvent (DMSO) peak of predominantly β-lactose powder is shown. * α-lactose content ± standard deviation. Error bars = standard deviation.*

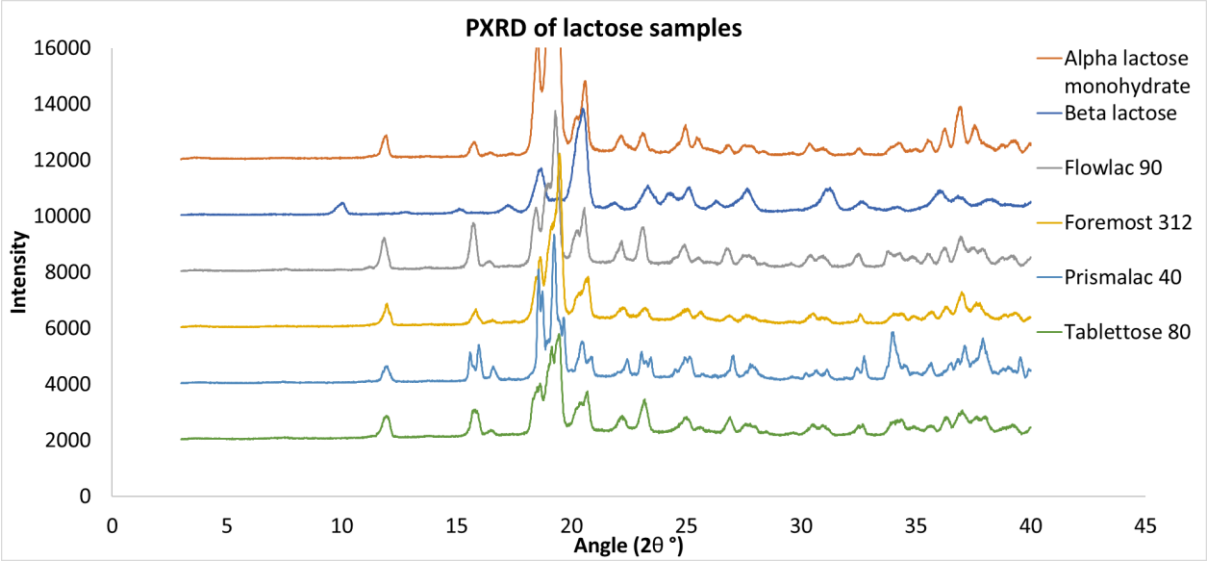


Figure 8 Overlay of PXRD spectra of lactose samples